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# Haemoglobin genotypes in cod (*Gadus morhua* L): their geographic distribution and physiological significance

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**ABSTRACT**
Haemoglobin polymorphism in cod (*Gadus morhua* L) has been investigated throughout the last 50 years. Field studies have shed light on the geographic distribution of the two common alleles (*Hbl*$^1$ and *Hbl*$^2$), and laboratory studies have shown effects of genotype on physiological traits such as growth, reproduction and hypoxia tolerance. The geographic distribution of alleles shows a correlation with temperature, with increasing frequency of *Hbl*$^1$ in warmer areas. This is likely due to temperature related differences in oxygen affinity of the three genotypes. We provide a general ecological introduction to cod haemoglobin polymorphism and a detailed discussion of physiological studies, particularly laboratory growth studies. Although differences in oxygen uptake are almost certainly a contributory mechanism to observed differences in traits such as growth rate, many other environmental, behavioural and social factors may also contribute, making it difficult to quantify the effect of *Hbl* either experimentally or in the field.

*Keywords:* cod, distribution, Gadus morhua, growth, haemoglobin, physiology

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# 1. Introduction

Haemoglobin (Hb) polymorphisms are common in many fish species such as salmon (*Oncorhynchus tshawytscha*), rainbow trout (*Oncorhynchus mykiss*) and carp (*Cyprinus carpio*) (Buhler and Shanks, 1959). In the early 1960s, the Danish geneticist Knud Sick was the first to describe Hb polymorphism in whiting (*Merlangius merlangus*) and Atlantic cod (*Gadus morhua*) (Sick, 1961). In cod, two haemoglobin loci were distinguished using agar gel electrophoresis; *HbI* and *HbII*. Gene products of *HbI*, predominating in adult cod, were polymorphic. Sick (1961) described a simple polymorphism at this locus with two alleles ($HbI^1$ and $HbI^2$) segregating into three easily distinguished genotypes; the two homozygotes *HbI-1/1* and *HbI- 2/2*, and the heterozygote *HbI-1/2*.

$HbI^1$ and $HbI^2$ are predominant throughout the species' range, but additional alleles exist (Sick, 1965*a*; Sick, 1965*b*; Frydenberg et al., 1965; Husebø et al., 2004; Verde et al., 2006; Borza et al., 2009; Halldórsdóttir and Árnason, 2009*a*; Halldórsdóttir and Árnason, 2009*b*; Andersen, 2012). Furthermore, studies using high-resolution techniques like IF (Isoelectric Focusing) have reported a previously undetected banding variability which probably represents both different alleles at *HbI* as well as other loci (Fyhn et al., 1994; Husebø et al., 2004). Among the reported rare genotypes, some appear to be more abundant in the far northern parts and others in the southern parts of the cod's range (Frydenberg et al., 1965; Sick, 1965*b*; Møller, 1968; Husebø et al., 2004; Andersen, 2012). Most recent papers do not show photos of gels with the actual *HbI* genotypes as they appear on different electrophoretic platforms. Therefore the respective interpretations of the *HbI* banding patterns cannot be cross-checked.

The genetic and molecular basis of the *HbI* protein variants and the link between the genotype and the physiological functions of the Hb gene products have been described in detail by Andersen et al. (2009) and Andersen (2012). For a detailed biochemical and molecular background to cod haemoglobin, we refer to the review by Andersen (2012).

The observed *HbI* genotypic distributions with $HbI^1$ decreasing from south to north (Sick, 1965*a*; Sick, 1965*b*; Frydenberg et al., 1965; Karpov and Novikov, 1980; Mork and Sundnes, 1984; Husebø et al., 2004; Andersen et al., 2009) have been associated with both geographic, between stock, differences in environmental temperatures and with individual, within stock, temperature preferences on a more local scale (Karpov and Novikov, 1980; Petersen and Steffensen, 2003; Behrens et al., 2012). Both explanations have been anchored to genotypic differences in physiological performance (e.g., Mork et al., 1983; Brix et al., 1998; Pörtner et al., 2001) and strongly suggest that cod haemoglobins are affected by natural selection (Kirpichnikov, 1981; Mork and Sundnes, 1985; McFarland, 1998; Andersen et al., 2009; Halldórsdóttir and Árnason, 2009*a*). Natural selection and behavioural habitat preferences are complementary explanations; environmental barriers may have led to genetic differentiation through local adaptations, and behaviour could potentially help maintain the variation. An example of this was recently demonstrated for the Icelandic cod stocks, where genetic differentiation (Pan I and microsatellite) was correlated with homing preferences for distinct spawning areas, i.e., preference for the warmer south-western areas or the cooler north-eastern areas (Pampoulie et al., 2011).

Physiological performance and aerobic capacity is determined by the ability to bind and unload oxygen. Haemoglobins with a high affinity bind $O_2$ at lower ambient levels, and the partial pressure at which haemoglobins are half saturated, $P_{50}$, is low. However, high $O_2$ affinity entails a matching reduction in the partial pressure of oxygen in the tissue for unloading of $O_2$ at the tissues.

Laboratory studies investigating the relationship between *HbI* polymorphisms, temperature preference and physiological performance are inconsistent. Differences in experimental design and methodology between studies may influence the outcome of the experiments, leading to results which in some cases appear to contradict the expectations arising from studies of geographical allele frequency distributions. Possible reasons for such inconsistencies need to be explored in order to move towards a more comprehensive understanding of the significance of

78 the *HbI* polymorphisms. Epigenetic and compensatory mechanisms influence physiological
79 performance, thereby making it difficult to distinguish the effect of haemoglobin from other
80 factors.

81 This review presents and discusses laboratory studies of the three main cod *HbI* genotypes,
82 examining the experimental design, methods and conclusions drawn. The observed allelic
83 distribution in natural populations is analysed in relation to temperature, and the physiological
84 performance of the different haemoglobin genotypes is discussed. Proposals for future research
85 are presented and evaluated.

86

87 ## 2. Distribution

88 *HbI* allele frequencies, which were related to latitude in the early studies (Sick, 1961*a*; Sick,
89 1961*b*; Frydenberg et al., 1965), have been corroborated in more recent reports (e.g., Karpov and
90 Novikov, 1980; Mork and Sundnes, 1984; Husebø et al., 2004; Andersen et al., 2009). However
91 it is evident that latitude *per se* has no effect on *HbI* allele frequency and that the observed clines
92 are due to water temperature (Pörtner et al., 2001).

93 Cod homozygous for $HbI^2$ are termed the *cold-water* type and cod homozygous for $HbI^1$ the
94 *warm-water* type (Frydenberg et al., 1965; Pörtner et al., 2001). With the possible exception of
95 Baltic Sea cod, which is also the most genetically divergent of all cod stocks (Mork et al., 1985),
96 the temperature related cline in cod *HbI* allele frequencies appears consistent in studies published
97 so far, and applies to both sides of the North Atlantic (Table 1).

98 In some areas, e.g., along the Norwegian coast (Møller, 1966; Møller, 1968; Dahle and Jørstad,
99 1993; Nordeide and Båmstedt, 1998; Wennevik et al., 2008) and in Icelandic waters (Jamieson
100 and Jónssen, 1971; Jamieson and Birley, 1989), intermingling of populations with different $HbI^1$
101 frequencies occurs during the spawning season, thus changing the prevailing *HbI* pattern. The
102 North East Arctic cod stock migrates from the cold Barents Sea to spawn in areas along the

Norwegian coast in February to April and this gives rise to a marked decrease in the *HbI*$^{1}$ frequency in those locations (Fig. 1). The noteworthy heterogeneity seen in the Icelandic waters (Table 1) may be explained by the existence of two genetically differentiated spawning components with one component preferring the warmer waters southwest of Iceland and the other the cooler waters in the northeast (Pampoulie et al., 2011). In other areas, where seasonal cod migrations are small and do not involve large temperature differences, e.g., the North Sea, there is no obvious seasonal change in local *HbI*$^{1}$ frequency (Fig. 2).

Overall, the *HbI* allele frequencies for the entire range of cod show an increase in *HbI*$^{1}$ with increasing temperature (Fig. 3, 4). However, the relationship between temperature and *HbI*$^{1}$ appears to be stepped, with the proportion of *HbI*$^{1}$ below 0.2 up to 4˚C, above 0.5 at temperatures above 8˚C and highly variable in the temperature range 5-8˚C. While this may in part be explained by the kind of seasonal differences in *HbI*$^{1}$ frequency observed at the Norwegian coast due to migration, it may also indicate that other genetic and environmental factors can influence the distribution of the *HbI* genotypes.

In some studies on the population structure of cod, authors have interpreted the geographic *HbI* allele frequency differences as signs of a complex genetic subdivision into separate and isolated populations (Dahle and Jørstad, 1992). However, if temperature is acting selectively then the utility of this locus as a neutral population marker is questionable (Mork et al., 1984*b*; Mork and Sundnes, 1985).

In the early juvenile stages of cod gene products of *HbII* predominate and *HbI* genes do not become active until the fish are 3-4 cm Fyhn et al. (1995). The authors hypothesized that the change in gene expression could be related to adaptation to a new environment, i.e., the shift from pelagic to demersal life style.

## 3. Physiological performance

Temperature is regarded as 'the ecological master factor' of ectothermal organisms (Brett, 1971) as it influences all aspects of their physiology (Lefrancois and Claireaux, 2003; Pörtner and Knust, 2007). Growth rate is maximised within a species-specific range of temperatures, often referred to as the optimal temperature for growth ($T_{opt}$), which for satiation fed, maturing and adult Atlantic cod kept at normoxic conditions ranges between 11 and 14°C (Jobling, 1983; Claireaux *et al*., 2000; Colosimo et al., 2003; Brix *et al*., 2004; Jordan et al., 2006). However $T_{opt}$ declines with increasing size in cod and is, thus, much higher in smaller fish (Pedersen and Jobling, 1989; Björnsson and Steinarsson, 2002). $T_{opt}$ is also reduced when food is limited because maintenance costs are higher at high temperatures (Brett, 1971). It is noteworthy that growth rates and condition factors increase with temperature over the actual thermal range occupied by cod in the wild and are highest in the warmest areas in which they occur (Dutil and Brander, 2003; Rätz and Lloret, 2003).

The reported $T_{opt}$ found in laboratory experiments with satiation fed cod is higher than observations from the field; a comprehensive tagging study encompassing thermal history data (>90 days) for more than 300 cod from 8 geographical areas demonstrated that maximum growth rates were obtained by fish having a mean thermal history of 8-10°C (Righton et al., 2010). This is probably due to differences in feed intake, but it is a mistake to regard it as evidence of food limitation, since the satiation fed experimental environment (or the environment of a fish farm) is an abnormal one.

### *3.1 Temperature preference*

In theory, the preferred temperature of a fish ($T_{pref}$) should reflect the optimal temperature for growth ($T_{opt}$), but thermoregulatory behaviour may not always ensure this (Jordan et al., 2006; Behrens et al., 2012). Basically, temperature preference strategies are extremely complex and vary with the physical environment (e.g., oxygen availability, Schurmann and Steffensen, 1992; Petersen and Steffensen, 2003) and internal factors / physiological state of the fish (e.g.,

digestive status (Gräns et al., 2010), size and age (Pedersen and Jobling, 1989; Glover et al., 1997; Björnsson and Steinarsson, 2002; Swain and Kramer, 1995; Gräns et al., 2010; Behrens *et al*., 2012)). Selecting a colder environment increases the blood oxygen affinity which increases the oxygen binding at the gills but may lower the oxygen unloading at the tissues. It also lowers the metabolism. These conditions favour energy conservation but not digestion, growth and fast swimming (Brett, 1971). Thus, there may be a trade-off between activities like feeding, digestion, predator avoidance and migration when selecting a thermal environment.

The geographic distribution of the two major haemoglobin alleles indicates that *HbI-1/1* is adapted to warm waters and *HbI-2/2* to cold waters (Sick, 1965*a*; Pörtner et al., 2001; Brix et al., 2004). Two separate studies have investigated the preferred temperatures of the *HbI* types, with partially discrepant results. Petersen and Steffensen (2003) observed a significant and rather pronounced difference between the two homozygotes with *HbI-1/1* showing preference for temperatures of 15.4±1.1°C and *HbI-2/2* for temperatures of 8.2±1.5°C when kept under normoxic conditions. During hypoxia (35% air saturation), $T_{pref}$ for *HbI-1/1* fish fell to 9.8±1.8°C, whereas no change was observed in *HbI-2/2* fish (Petersen and Steffensen, 2003). The authors, however, hypothesized that a similar decrease in $T_{pref}$ of *HbI-2/2* might be observed if the oxygen levels were further reduced (<33%).

The observed dissimilarities in $T_{pref}$ was recently confirmed by Behrens et al. (2012); *HbI-2/2* preferred temperatures of 8.9±0.2°C, consistent with findings by Petersen and Steffensen (2003), but *HbI-1/1* preferred substantially lower temperatures (11.0±0.6°C) than reported by Petersen and Steffensen (2003), yet still significantly higher than the value for *HbI-2/2* cod within the same study. The discrepancy between the two studies might be due to different methodologies, shuttle box versus an annular preference chamber. Furthermore, based on PCR and SNPs (single nucleotide polymorphisms), Behrens et al. (2012) observed that 17% of the experimental fish had recombinant haplotypes that were not compatible with any of the *HbI* genotypes, and it is possible that these haplotypes disturbed the overall pattern of temperature preference in both studies. Similar biases have been emphasized by other studies (Brix et al., 1998; Husebø et al.,

2004; Andersen et al., 2009; Borza et al., 2009). Heterozygotes display less distinct temperature preferences, not significantly different from any of the homozygotes, and with greater inter-individual variation (Behrens et al. 2012). Furthermore, heterozygotes have a high degree of plasticity in their expression of the two haemoglobins (Brix *et al*., 2004; Andersen et al., 2009; Borza *et al*., 2009) and are, thus, likely to thrive in a broader thermal range.

*3.2 Oxygen transport*

The earliest study on the *in vitro* oxygen affinity of the three common cod *HbI* genotypes was performed on intact erythrocytes and showed highest oxygen affinities for *HbI-2/2* and *HbI-1/1* at temperatures below and above 15°C, respectively (Karpov and Novikov 1980). These genotype-specific oxygen affinities have later been confirmed by other *in vitro* studies performed on haemolysates, although with less pronounced differences (Brix et al., 1998; Pörtner et al., 2001). However, in an *in vitro* study of haemolysates by McFarland (1998), a slightly different result was obtained: in fish acclimated to 10 and 16˚C, no genotypic differences were found, but at an acclimation of 4˚C, *HbI-2/2* had a higher $O_2$ affinity. Similarly, when 10˚C acclimated fish were exposed to 4 and 16˚C, the *HbI-2/2* had higher $O_2$ affinity. If the fish were allowed no acclimation time prior to experiments, a steady decrease in the affinity of *HbI-2/2* between 4 to 16 ˚C was evident. The $O_2$ affinity of *HbI-1/1* was shown to be less temperature sensitive, and in later molecular studies this type is referred to as the temperature-insensitive haplotype (Andersen et al., 2009; Andersen, 2012).

Haematocrit, the ratio between erythrocytes and blood plasma, is also relevant in relation to physiological performance as more erythrocytes will enhance the blood oxygen carrying capacity. Mork and Sundnes (1984) investigated haematocrit in juvenile and adult cod from a Norwegian fjord and found higher haematocrit values in males homozygous for $HbI^2$, but no differences between genotypes for females. Their results were later supported by McFarland (1998) who found higher haematocrit values in adult *HbI-2/2* at 5 and 10˚C, regardless of sex. In

addition, when fish were allowed a period of 4 weeks acclimation prior to the blood sampling, the *HbI-2/2* type was also observed to have a higher haemoglobin concentration compared to the *HbI-1/1* type (McFarland, 1998).

Despite small dissimilarities in the above mentioned studies, it appears that *HbI-2/2* has superior oxygen transport capabilities at low ambient temperature especially under hypoxic conditions, which is also reflected in the distribution of the $HbI^2$ allele.

### *3.3. Hypoxia tolerance*

Having a high oxygen affinity is considered advantageous for individuals encountering hypoxic conditions (Brix *et al*., 1998). The critical oxygen saturation ($S_{crit}$) can be defined as the $O_2$ level where a fish can no longer maintain an oxygen uptake that supports the standard metabolic rate (SMR; the oxygen uptake of resting, fasting fish) and can as such be used as a measure of hypoxia tolerance. Few studies have investigated the response of the *HbI* genotypes to hypoxia, and with ambiguous results. The first study was conducted on wild-caught adult cod that were allowed at least 4 weeks of acclimation to 4, 10 or 16˚C (McFarland, 1998). No differences were observed in $S_{crit}$ values at 10 and 16˚C, but at 4˚C, *HbI-2/2* had a lower $S_{crit}$. This also coincided with a significantly lower SMR in *HbI-2/2* at this temperature. When fish acclimated to 10˚C were exposed to 4 and 16˚C, the lowest $S_{crit}$ was seen in *HbI-2/2* and *HbI-1/1*, respectively. McFarland (1998) concluded that *HbI-2/2* is more tolerant to hypoxia but only when the fish are in a resting, fasting state which is supporting their SMR.

Two separate studies on juvenile offspring from wild-caught cod acclimated for either 1 or 5 weeks, failed to find any differences between the two *HbI* types in the avoidance of hypoxic environments, ventilation rates or cortisol levels (Skjæråsen *et al*., 2008; Gamperl *et al*., 2009). However, Gamperl et al., (2009) observed *HbI-1/1* to have a lower $S_{crit}$ than the other homozygote, suggesting that this genotype as opposed to the above-mentioned findings is more tolerant of hypoxia (Gamperl et al., 2009). Conversely, Methling et al. (2010) observed higher

plasma cortisol and lactate levels in *HbI-1/1*, after hypoxia exposure indicative of an increased level of stress in wild-caught juveniles allowed 3 weeks of acclimation. Petersen and Steffensen (2003) also hypothesized that *HbI-2/2* is more tolerant to hypoxia based on their observation that this genotype did not shift its $T_{pref}$ at hypoxia. Altogether the above suggests that stress response and the associated metabolic adjustments may be *HbI*-dependent – although it is not yet clear how and to what degree.

The apparent lack of a clear correlation between oxygen affinity and hypoxia tolerance may be partly due to the fact that *in vitro* studies of $O_2$ affinity do not take into account the possibility of *in vivo* compensatory mechanisms. These can be of behavioural character e.g., avoidance of hypoxic habitats, increased ventilation, reduced activity levels or cessation of feeding (McFarland, 1998; Gamperl et al., 2009), and/or related to cellular compensatory control of Hb-$O_2$ affinity. Here organic phosphates (in fish mainly ATP and GTP) can be of major adaptive significance as these, when bound to the Hb, lower the O2 affinity (Weber & Jensen 1988; Jensen et al., 1998). Thus, reducing the level of red blood cell organic phosphates is a mean to increase blood oxygen affinity during hypoxia (Val, 2000; Nikinmaa, 2001), which has also been documented in cod by McFarland (1998), who found a decrease in $P_{50}$ concurrently with a decrease in ATP and GTP levels, thereby increasing the oxygen uptake in the blood. In conclusion, compensatory mechanisms are likely to reduce the genotypic differences, indirectly suggesting that the genotypes are in fact exceptionally flexible.

### *3.4 Growth*

The growth rate and growth potential of a fish is determined by its developmental stage, genotype and environment (e.g., food availability and quality, temperature and oxygen availability). Separating the effects of each of these factors is ultimately impossible, because they interact antagonistically or synergistically, and this is one highly plausible reason for the inconsistent and contradictory results obtained in studies dealing with growth of cod with

different haemoglobin genotypes. Conclusions drawn from comparing length and weight data from the field are also restricted by limited knowledge of the environmental and physiological history of the fish. Field estimates of growth show general trends for individuals or populations living in a particular area and under a more or less well-known set of environmental conditions. Differences in growth rate between cod stocks were documented as early as the 1930s, when the Norwegian coastal cod were observed to grow faster and mature earlier than the Northeast Arctic cod (Hylen, 1933; Rollefsen, 1934). However, a common-garden experiment by Godø and Moksness (1987) demonstrated that fish from these two stocks grow and mature at the same rates under identical environmental conditions and this is confirmed by other studies (e.g., Taranger et al., 2006).

Some field studies, comparing lengths of cod with different genotypes, have shown growth-related differences whereas others have not (Sick, 1965*a*; Gjøsæter et al., 1992; Jørstad and Nævdal, 1994). Higher growth rates of *HbI-2/2* have been reported in colder areas with temperatures closer to the genotype's $T_{pref}$ (Mork et al., 1983; Mork and Sundnes, 1984; Mork et al., 1984*a*; Mork et al., 198b*a*). For instance, in the Baltic Sea where the temperature and $HbI^1$ frequency decrease from west to east, a similar decrease in length and weight of *HbI-1/1* is seen (Pörtner et al., 2001). In the southern part of the North Sea, where $HbI^1$ is the predominant allele, *HbI-1/1* fish are generally larger than *HbI-2/2* fish (Pörtner et al., 2001). In conclusion, *HbI-2/2* appears to be superior to *HbI-1/1* at colder temperatures, and vice-versa (Mork and Sundnes, 1984).

Comparing results and drawing conclusions from laboratory studies is made difficult by the diversity of methodology, duration, acclimatization period, tank environment, temperature regime, photoperiod, feed, and so forth. We assume that *HbI* genotypes, according to Sick (1961), were correctly identified by the various electrophoretic techniques employed in the different studies, and, thus, that the observed inconsistencies are caused by other issues. An overview of these as well as other physiology studies is given in Table 2.

The most commonly drawn conclusion from laboratory studies is that no *HbI* genotypic differences in growth exist (Colosimo et al., 2003; Gjerde et al., 2004; Jørstad et al., 2006; Jordan et al., 2006). The varieties of experimental design may, nevertheless, mask the actual effects of *HbI*. Two studies show significant differences; Nævdal et al. (1992) found higher growth rates for *HbI-2/2* at all temperatures (6-14˚), whereas Imsland *et al*. (2004) observed highest growth rates in *HbI-2/2* at high temperatures (13-16˚C) and in *HbI-1/1* at low temperatures (7˚C). The results obtained in the latter study deviates from all earlier findings regarding allelic distribution, $T_{pref}$ and $O_2$ affinity. The difference in growth could possibly be caused by a sibling or pedigree effect as the experimental animals were offspring from wild-caught fish and if a skewed proportion of the juveniles originate from the same parents, they are likely to be more similar in physiological performance. Glover et al. (1997) observed a tendency for higher growth in *HbI-2/2* at 12˚C and in *HbI-1/1* at higher temperatures, but the results were not significant. A fourth study, showed the opposite pattern: *HbI-1/1* grew faster than *HbI-2/2* when fed in excess (Gamperl et al., 2009). Reducing the amount of food removed this discrepancy (Gamperl et al., 2009).

Most of the laboratory studies selected juveniles randomly without considering the family relations. In a more recent study conducted by Gamperl *et al*. (2009) two heterozygotes were crossed and the offspring used for the experiments – thereby reducing the amount of unknown genetic factors potentially influencing the experiments. In this study *HbI-1/1* was observed to grow faster throughout the first 9 months when kept at 10˚C and fed in excess. Gjerde et al. (2004) also used full-sib families in their study, and although they did not find differences in growth, they observed a relatively high degree of heritability of growth (body weight), i.e., siblings resembled each other. They hypothesized that additive genetic effects (other than haemoglobin) likely influenced the results. On the contrary, Jørstad et al. (2006) were unable to distinguish between effects of genotype and family in their experiments. To disentangle this, future experiments should be based on well-defined families preferably with heterozygotic parents.

In most growth studies fish are fed *ad libitum*, simulating commercial fish farm conditions. However, acknowledging that food is rarely found in excess in nature and further that fish behave differently according to their personality (Adriaenssens and Johnsson, 2011), keeping experimental animals under such artificial conditions may likely produce results not comparable to what is observed in the wild. Salvanes and Hart (2000) investigated feeding behaviour in cod with different haemoglobin genotypes and found that *HbI-2/2* had the highest prey capture success and were generally the first to take prey. It was, hence, concluded that in situations where fish have to compete for food, cod homozygous for $HbI^2$ have an advantage. Exposing experimental animals to more natural feeding conditions would presumably result in more pronounced behavioural differences, mimicking those seen in nature.

Another issue in relation to behaviour is that genotypes are often held together randomly with no consideration of haemoglobin composition. If a tank is dominated by fish homozygous for $HbI^2$, which are known to be more competitive, this is likely to influence the results. Salvanes and Hart (2000) used two types of experimental setup; one in which three fish (one of each genotype) where held together and another in which four fish were chosen at random. The "group" factor was shown to have a significant effect on feeding behaviour, emphasizing the importance of knowing and if necessary manipulating the haemoglobin composition in tanks. Additionally, the experiments should be replicated to control for "tank effects". In this study *HbI*-2/2 was shown to be superior, however other combinations of haemoglobin genotypes may result in a shift in competitive superiority (Salvanes and Hart, 2000). In nature, cod of different genotypes usually co-exist, and it would, thus, be interesting to test for competitive performance within different groupings. Only in one of the growth studies were the genotypes reared separately (Jørstad et al., 2006) and the results did not show any differences in growth.

Considering that cod show plasticity in their expression of the different haemoglobin components (Brix *et al*., 2004; Andersen et al., 2009; Borza *et al*., 2009), an acclimation period prior to temperature experiments will likely influence the growth rate and perhaps impede the actual haemoglobin effects. In addition to this, epigenetic compensation mechanisms controlled

by other loci may disguise these effects further (Stearns, 1992; Donelson et al., 2012). The length of the acclimation period varies significantly between studies; from a few days (Jordan et al., 2006, Gamperl et al., 2009) up to several months (Nævdal et al., 1992; Imsland et al., 2004). A long acclimation period seem to reduce the differences between the genotypes, acclimation time should be kept at a minimum. However, this raises the problem that stressed fish most likely will not feed voluntarily, which consequently influences the results. A short acclimation period may, in theory, favour the more stress-resistant *HbI* type.

In most of the experimental studies, 2$^{nd}$ generation cod i.e., offspring from wild-caught fish, were used. Collection of the parental fish from the wild differed between the studies both with regard to the time of year and fishing method (trawl, long line, etc.). This may have had large impacts on the experiments described previously due to differences in the environment inhabited by the parent fish. Seasonal changes such as migration to and from spawning / feeding areas as well as gear type will influence the "selection of fish", likely biasing the sampling in a particular, however, largely unknown direction. The conditions experienced in captivity are far from natural, despite attempts to make them so i.e., matching the photoperiod and temperature experienced by the fish in the wild. Lack of predators, limited space for movement and constant feeding regimes constitute some of the main differences. These will inevitably result in some degree of acclimatization, and this phenotypic plasticity may be reflected in the offspring. Studies, in which offspring from farmed fish have been used, may also be a source of variation as these fish are likely to have adapted to the farmed conditions through several generations.

## 4. Discussion and Conclusion

The observed latitudinal cline in *HbI*$^{1}$ frequency is widely believed to be the result of selection with cod homozygous for *HbI*$^{1}$ and *HbI*$^{2}$ preferring warmer and colder waters, respectively. This relationship is also reflected in the oxygen affinity, which is highest for *HbI-2/2* at lower temperatures and vice versa for *HbI-1/1*. The heterozygote is somewhere in-between and is likely

to be the most flexible of the three common genotypes as it possesses both haemoglobins in equal quantities.

Despite the clear association between the haemoglobin genotype and the preferred temperature and oxygen affinity, correlations with other physiological traits such as thermal tolerance and growth are less obvious. This may be due to both the difficulty of estimating the influence of a single factor (*HbI*) when a number of factors interact, and also to limitations of experimental designs.

Fish growth is affected by multiple environmental and genetic factors. Laboratory experiments allow for a certain amount of control and reduce some of the influential factors but, nevertheless, introduce new problems such as tank and sibling effects. Experimenters also have to deal with fish personality (competition, aggression, and cooperative behaviour), acclimation time and feeding regime which are all likely to affect the outcome, although often in unpredictable ways.

Having emphasized all the problems and pitfalls with different experimental setups, it seems appropriate to outline what a *good* approach to study *HbI* genotypic growth rates is. Experiments should be performed on full-siblings by crossing of two wild-caught heterozygotes. Several families should be included in the study allowing for differences in phenotypic plasticity due to variation in the environment inhabited by the families (northern/southern latitudes, different depth regimes, coastal/offshore, etc.). Full-siblings within each family should be exposed to different temperature regimes and oxygen saturation levels, and if possible also different feeding regimes (*ad libitum*/minimum). The tanks should ideally be deep, allowing for vertical migrations, and the haemoglobin composition should either be balanced or differ between tanks to test for effects of competition (and fish behaviour in general). Acclimation periods should be kept at a minimum, yet enabling all the fish to fully recover from stress, to allow for the actual *HbI* genotypic variation to manifest itself. Fish farm facilities would probably be the most suitable for such a large-scale experiment which is, of course, very labour- and costs consuming.

An enhanced understanding of cod haemoglobins and their influence on various physiological traits will help both in understanding genome-environment interactions in natural cod populations, and be beneficial for optimizing the conditions for cod rearing in aquaculture. The knowledge will give better insight into processes affected by climate change, making prediction of the response of cod with different haemoglobin genotypes more reliable. In aquaculture, the knowledge can be used to improve facility settings to fit a particular *HbI* genotype, potentially maximizing the yield.

The candidate gene approach (single nucleotide polymorphisms, SNPs) may offer some promising prospects in relation to our understanding of natural selection working on specific genes and resulting in certain life-history traits and strategies (Hemmer-Hansen et al. 2011). Polymorphic candidate genes of known function can link genetic variation with individual differences in physiological functions (Hemmer-Hansen et al. 2011). For example, an association between particular SNPs and growth has been demonstrated in Arctic charr (*Salvelinus alpinus)* (Tao and Boulding, 2003). Efforts to relate genetic variation with physiological traits using SNPs have also been made for Atlantic cod (Moen et al., 2008; Nielsen et al., 2009; Hubert et al., 2010). However, whether the candidate gene approach can shed light on the effects of different haemoglobins and potential cooperation between haemoglobin loci is yet to be investigated.

In conclusion, it is important to keep in mind that haemoglobin is not the only factor influencing the physiology of cod – epistatic interactions as well as pleiotrophic effects are likely to be involved in shaping the various phenotypes observed in different experimental situations. To establish an experimental design, which can disentangle the various genetic sources of variation and their interactions is a very challenging goal, thus the underlying complexities must be kept in mind when interpreting experimental results.

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# References


Adriaenssens, B., Johnsson, J.I. 2011. Shy trout grow faster: exploring links between personality and fitness-related traits in the wild. Behav. Ecol. 22 (1), 135-43.

Andersen, Ø., Wetten, O.F., De Rosa, M.C., Andre, C., Alinovi, C.C., Colafranceschi, M., Brix, O., Coloismo, A. 2009. Haemoglobin polymorphisms affect the oxygen-binding properties in Atlantic cod populations. P. Roy. Soc. B – Biol. Sci. 276, 833-841.

Andersen, Ø. 2012. Hemoglobin polymorphisms in Atlantic cod – A review of 50 years of study. Mar. Genomics 8, 59-65.

Behrens, J., Gräns, A., Therkildsen, N.O., Neuenfeldt, S., Axelsson, M. 2012. Correlations between haemoglobin type and temperature preference of juvenile Atlantic cod *Gadus morhua*. J. Exp. Mar. Biol. Ecol. 413, 71-77.

Björnsson, B., Steinarsson, A. 2002. The food-unlimited growth rate of Atlantic cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. 59, 494-502.

Borza, T., Stone, C., Gamperl, A.K. and Bowman, S. 2009. Atlantic cod (*Gadus morhua*) hemoglobin genes: multiplicity and polymorphism. BMC Genet. 10: 51.

Brett, J.R. 1971. Energetic responses of salmon to temperature. A study of some thermal regulations in the physiology and freshwater ecology of Sockeye salmon (*Oncorhynchus nerka*). Am. Zool. 11 (1), 99-113.

Brett, J.R. 1979. Environmental factors and growth. *In* Fish Physiology vol. 8. Bioenergetics and growth. Edited by Hoar, W.S., Randall, D.J. and Brett, J.R. Academic Press.

Brix, O., Forås, E., Strand, I. 1998. Genetic variation and functional properties of Atlantic cod hemoglobins: Introducing a modified tonometric method for studying fragile hemoglobins. Comp. Biochem. Physiol. A 119, 575-583.

441 Brix, O., Thorkildsen, S., Colosimo, A. 2004. Temperature acclimation modulates the oxygen
442 binding properties of the Atlantic cod (*Gadus morhua* L.) genotypes HbI*1/1, HbI*1/2, and
443 HbI*2/2 by changing the concentrations of their major hemoglobin components (results from
444 growth studies at different temperatures). Comp. Biochem. Physiol. A 138, 241-251.

445 Buhler, D.R., Shanks, W.E. 1959. Multiple hemoglobins in fishes. Science 129 (3353), 899-900.

446 Claireaux, G., Webber, D.M., Lagardere, J.P., Kerr, S.R. 2000. Influence of water temperature
447 and oxygenation on the aerobic metabolic scope of Atlantic cod (*Gadus morhua*). J. Sea Res. 44,
448 257-265.

449 Colosimo, A., Giuliani, A., Maranghi, F., Brix, O., Thorkildsen, S., Fischer, T., Knust, R.,
450 Pörtner, H.O. 2003. Physiological and genetical adaptation to temperature in fish populations.
451 Cont. Shelf Res. 23, 1919-28.

452 Dahle, G., Jørstad, K.E. 1993. Haemoglobin variation in cod – a reliable marker for Arctic cod
453 (*Gadus morhua* L.). Fish. Res. 16, 301-311.

454 Dahle, G., Jørstad, K.E., Rusaas, H.E., Otterå, H. 2006. Genetic characteristics of broodstock
455 collected from four Norwegian coastal cod (*Gadus morhua*) populations. ICES J. Mar. Sci. 63,
456 209-15.

457 Donelson, J.M., Munday, P.L., McCormick, M.I., Pitcher, C.R. 2012. Rapid transgenerational
458 acclimation of a tropical reef fish to climate change. Nat. Clim. Chan. 2 (1), 30-32.

459 Dutil, J.D., Brander, K.M. 2003. Comparing productivity of North Atlantic cod (*Gadus morhua*)
460 stocks and limits to growth production. Fish. Oceanogr. 12, 502–512.

461 Frydenberg, O., Møller, D., Nævdal, G., Sick, K. 1965. Haemoglobin polymorphism in
462 Norwegian cod populations. Hereditas-Genetisk A. 53, 257-271.

463 Fyhn, U.E.H., Brix, O., Nævdal, G., Johansen, T. 1994. New variants of the haemoglobins of
464 Atlantic cod - A tool for discriminating between coastal and Arctic cod populations. Int. Co.
465 Explor. Sea, Copenhagen K.

466 Fyhn, U.E.H., Johansen, T., Nævdal, G., Thorkildsen, S. 1995. Ontogeny of haemoglobin
467 polymorphism in cod. J. Fish Biol. 47, 56-60.

468 Gamperl, A.K., Busby, C.D., Hori, T.S.F., Afonso, L.O.B., Hall, J.R. 2009. Hemoglobin
469 genotype has minimal influence on the physiological response of juvenile Atlantic cod (*Gadus*
470 *morhua*) to Environmental Challenges. Physiol. Biochem. Zool. 82, 483-494.

471 Gjerde, B., Terjesen, B.F., Barr, Y., Lein, I., Thorland, I. 2004. Genetic variation for juvenile
472 growth and survival in Atlantic cod (*Gadus morhua*). Aquaculture 236, 167-177.

473 Gjøsæter, J. Jørstad, K., Nævdal, G., Thorkildsen, S. 1992. Genotype distributions of cod from
474 the Norwegian Skagerrak coast. Sarsia 76, 255-259.

475 Glover, K., Nyhammer, G., Nævdal, G., Otterlei, E., Thorkildsen, S. 1997. Studies on genotype
476 dependent growth in juvenile cod (*Gadus morhua*) reared at different temperatures and light
477 regimes. Report series, 1997 (8). Department of Fisheries and Marine Biology, University of
478 Bergen, Norway, 13 pp.

479 Godø, O.R., Moksness, E. 1987. Growth and maturation of Norwegian coastal cod and Northeast
480 Arctic cod under different conditions. Fish. Res. 5 (2-3), 235-42.

481 Gräns, A., Olsson, C., Pitsillides, K., Nelson, K.H.E., Cech, J.J. Jr., Axelsson, M. 2010. Effects
482 of feeding on thermoregulatory behviours and gut blood flow in white sturgeon (*Acipenser*
483 *transmontanus*) using biotelemetry in combination with standard techniques. J. Exp. Biol. 213,
484 3198-3206.

485 Halldórsdóttir, K., Árnason, E. 2009*a*. Multiple linked β and α globin genes in Atlantic cod: A
486 PCR based strategy of genomic exploration. Mar. Genomics 2, 169-81.

487 Halldórsdóttir, K., Árnason, E. 2009*b*. Organization of a β and α globin gene set in the teleost
488 Atlantic cod, *Gadus morhua*. Biochem. Genet. 47, 817-830.

489 Hemmer-Hansen, J., Nielsen, E.E., Meldrup, D., Mittelholzer, C. 2011. Identification of single
490 nucleotide polymorphisms in candidate genes for growth and reproduction in a nonmodel
491 organism: the Atlantic cod, *Gadus morhua*. Mol. Ecol. Resour. 11 (Suppl. 1), 71-80.

492 Hubert, S., Higgins, B., Borza, T., Bowman, S. 2010. Development of a SNP resource and a
493 genetic linkage map for Atlantic cod (*Gadus morhua*). BMC Genomics 11: 191.

494 Husebø, A., Imsland, A.K., Nævdal, G. 2004. Haemoglobin variation in cod: a description of
495 new variants and their geographical distribution. Sarsia 89, 369-378.

496 Hylen, A. 1964. Coastal cod and skrei in the Lofoten area. FiskDir. Skr. Ser. HavUnders. 13 (7),
497 27-42.

498 ICES. www.ices.dk.

499 Imsland, A.K., Jónsdóttir, Ó.D.B. 2002. Is there a genetic basis to growth in Atlantic cod? Fish
500 Fish. 3, 36-52.

501 Imsland, A.K., Foss, A., Nævdal, G., Johansen, T., Folkvord, A., Stefansson, S.O., Jonassen, T.
502 M. 2004. Variations in growth in haemoglobin genotypes of Atlantic cod. Fish Physiol.
503 Biochem. 30, 47-55.

504 Jamieson, A., Jónssen, J. 1971. The Greenland component of spawning cod at Iceland. Rapports
505 et Procés-Verbaux des Réunions 161, 65-72. Cons. Int. Explor. Mer.

506 Jamieson, A., Otterlind, G. 1971. The use of cod blood protein polymorphisms in the Belt Sea,
507 the Sound and the Baltic Sea. Rapports et Procés-Verbaux des Réunions 161, 55-59. Cons. Int.
508 Explor. Mer.

509 Jamieson, A., Thompson, D. 1972. Blood proteins in North Sea cod (*Gadus morhua* L.). XIIth
510 European Conference on Animal Blood Groups Biochemical Polymorphism, Bp., 1972, 585-91.

511 Jamieson, A., Birley, A.J. 1989. The demography of a haemoglobin polymorphism in the
512 Atlantic cod, *Gadus morhua* L. J. Fish Biol. 35 (suppl. A), 193-204.

513 Jensen, F.B., Fago, A., Weber, R.E. 1998. Hemoglobin structure and function. *In* Fish
514 Respiration (Perry, S. F. and Tufts, C. R., Eds.), pp. 1-40. Academic Press, San Diego.

515 Jobling, M. 1983. Growth-studies with fish - Overcoming the problems of size variation. J. Fish
516 Biol. 22, 153-157.

517 Jordan, A.D., Lampe, J.F., Grisdale-Helland, B., Helland, S.J., Shearer, K.D., Steffensen, J.F.
518 2006. Growth of Atlantic cod (*Gadus morhua* L.) with different haemoglobin subtypes when
519 kept near their temperature preferenda. Aquaculture 257, 44-52.

520 Jørstad, K.E., Nævdal, G. 1994. Studies on associations between genotypes and growth rate in
521 juvenile cod. ICES Mar. Sc. 198, 671-75.

522 Jørstad, K.E., Karlsen, O., Svasand, T., Otterå, H. 2006. Comparison of growth rate among
523 different protein genotypes in Atlantic cod, *Gadus morhua*, under farmed conditions. ICES J.
524 Mar. Sci. 63 (2), 235-45.

525 Karpov, A.K., Novikov, G.G. 1980. Hemoglobin alloforms in the cod *Gadus morhua*,
526 gadiformes Gadidae. Their functional features and distribution in populations. Vop. Ikhtiol. 20,
527 823-827.

528 Karpov, A.K., Osinov, A.G., Novikov, G.G. 1984. Protein polymorphism in Atlantic cod, *Gadus*
529 *morhua* (Gadidae), of the White Sea. J. Ichthyol. 24, 71-79.

530 Kirpichnikov, V.S. 1981. Genetic bases of fish selection. Springer-Verlag, Berlin, 410 pp.

Lefrancois, C., Claireaux, G. 2003. Influence of ambient oxygenation and temperature on metabolic scope and scope for heart rate in the common sole *Solea solea*. Mar. Ecol.-Prog. Ser. 259, 273-284.

McFarland, S. 1998. Biochemical and physiological adaptations of Haemoglobin-I genotypes of Atlantic cod, *Gadus morhua* L. PhD thesis, The University of Birmingham.

Methling, C., Aluru, N., Vijayan, M.M., Steffensen, J.F. 2010. Effect of moderate hypoxia at three acclimation temperatures on stress responses in Atlantic cod with different haemoglobin types. Comp. Biochem. Physiol. A 156, 485-490.

Moen, T., Hayes, B., Nilsen, F., Delghandi, M., Fjalestad, K.T., Fevolden, S.E., Berg, P.R., Lien, S. 2008. Identification and characterisation of novel SNP markers in Atlantic cod: Evidence for directional selection. BMC Genet. 9: 18.

Mork, J., Sundnes, G. 1984. Hemoglobin polymorphism in *Gadus Morhua*: Genotypic differences in hematocrit. Helgolander Meeresun. 38, 201-206.

Mork, J., Sundnes, G. 1985. Hemoglobin polymorphism in Atlantic cod (*Gadus morhua*): Allele frequency variation between yearclasses in a Norwegian fjord stock. Helgolander Meeresun. 39, 55-62.

Mork, J., Giskeødegaard, R., Sundnes, G. 1983. The haemoglobin Polymorphism in *Gadus morhua*: genotypic differences in maturing age and within-season gonad maturation. Helgolander Meeresun. 36, 313-322.

Mork, J., Giskeødegaard, R., Sundnes, G. 1984*a*. The haemoglobin polymorphism in Atlantic cod (*Gadus morhua* L.); genotypic differences in somatic growth and in maturing age in natural populations. Flödevigen Rapportserie 1, 721-32.

553 Mork, J., Giskeødegaard, R., Sundnes, G. 1984*b*. Population genetic studies in cod (*Gadus*
554 *morhua* L.) by means of the haemoglobin polymorphism: observations in a Norwegian coastal
555 population. FiskDir. Skr. (Havundersøgelser) 17, 449-71.

556 Mork, J., Ryman, N., Ståhl, G., Utter, F., Sundnes, G. 1985. Genetic variation in Atlantic cod
557 (*Gadus morhua*) throughout its range. Can. J. Fish. Aquat. Sci. 42, 1580-87.

558 Møller, D. 1966. Genetic differences between cod groups in the Lofoten area. Nature 19, 824.

559 Møller, D. 1968. Genetic diversity in spawning coda long the Norwegian coast. Hereditas 60, 1-
560 32.

561 Nielsen, E.E., Hemmer-Hansen, J., Poulsen, N.A., Loeschcke, V., Moen, T. Johansen, T.,
562 Mittelholzer, C., Taranger, G.L., Ogden, R., Carvalho, G.R. 2009. Genomic signatures of local
563 directional selection in a high gene flow marine organism; the Atlantic cod (*Gadus morhua*).
564 BMC Evol. Biol. 9: 276.

565 Nikinmaa, M. 2001. Haemoglobin function in vertebrates: evolutionary changes in cellular
566 regulation in hypoxia. Resp. Physiol. 128 (3), 317-29.

567 Nordeide, J.T., Båmstedt, U. 1998. Coastal cod and north-east Arctic cod – do they mingle at the
568 spawning grounds in Lofoten? Sarsia 83 (5), 373-79.

569 Nævdal, G., Folkvord, A., Otterlei, E., Thorkildsen, S. 1992. Growth-rate related to genotype of
570 0-group cod at 3 environmental temperatures. Sarsia 77, 71-73.

571 Pampoulie. C., Ruzzante, D.E., Chosson, V., Jörundsdóttir, T.D., Taylor, L., Thorsteinsson, V.,
572 Daníelsdóttir, A.K., Marteinsdóttir, G. 2011. The genetic structure of Atlantic cod (*Gadus*
573 *morhua*) around Iceland: insight from microsatellites, the Pan I locus, and tagging experiments.
574 Can. J. Fish. Aquat. Sci. 63 (12), 2660-74.

575 Pedersen, T., Jobling, M. 1989. Growth rates of large, sexually mature cod, *Gadus morhua*, in
576 relation to condition and temperature during an annual cycle. Aquaculture 81, 161-168.

577 Petersen, M.F., Steffensen, J.F. 2003. Preferred temperature of juvenile Atlantic cod *Gadus*
578 *morhua* with different haemoglobin genotypes at normoxia and moderate hypoxia. J. Exp. Biol.
579 206, 359-364.

580 Pörtner, H.O., Berdal, B., Blust, R., Brix, O., Colosimo, A., De Wachter, B., Giuliani, A.,
581 Johansen, T., Fischer, T., Knust, R., Lannig, G., Naevdal, G., Nedenes, A., Nyhammer, G.,
582 Sartorisa, F.J., Serendero, I., Sirabella, P., Thorkildsen, S., Zakhartsev, M. 2001. Climate
583 induced temperature effects on growth performance, fecundity and recruitment in marine fish:
584 developing a hypothesis for cause and effect relationships in Atlantic cod (*Gadus morhua*) and
585 common eelpout (*Zoarces viviparus*). Cont. Shelf Res. 21, 1975-1997.

586 Pörtner, H.O., Knust, R. 2007. Climate change affects marine fishes through the oxygen
587 limitation of thermal tolerance. Science 315, 95-97.

588 Righton, D.A., Andersen, K.H., Neat, F., Thorsteinsson, P.S., Svedäng, H., Michalsen, K.,
589 Hinrichsen, H.H., Bendall, V., Neuenfeldt, S., Wright, P., Jonsson, P., Huse, G., van der Kooij,
590 J., Mosegaard, H., Hüssy, K., Metcalfe, J. 2010. Thermal niche of Atlantic cod *Gadus morhua*:
591 limits, tolerance and optima. Mar. Ecol.-Prog. Ser. 420, 1-13.

592 Rollefsen, G. 1934. The cod otolith as a guide to race, sexual development and mortality. Rapp.
593 P.V. Reun. Cons. Int. Explor. Mer. 88 (2), 1-15.

594 Rätz, H.J., Lloret, J. 2003. Variation in fish condition between Atlantic cod (*Gadus morhua*)
595 stocks, the effect on their productivity and management implications. Fish. Res. 60, 369–380.

596 Salvanes, A.G.V., Hart, P.J.B. 2000. Is individual variation in competitive peformance of reared
597 juvenile cod influenced by haemoglobin genotype? Sarsia 85, 265-74.

598 Schurmann, H., Steffensen, J.F. 1992. Lethal oxygen levels at different temperatures and the
599 preferred temperature during hypoxia of the Atlantic cod, *Gadus morhua* L. J. Fish Biol. 41, 927-
600 934.

601 Sick, K. 1961. Haemoglobin polymorphism in fishes. Nature 192, 894-896.

602 Sick, K. 1965*a*. Haemoglobin polymorphism of cod in Baltic and Danish Belt Sea. Hereditas-
603 Genetisk A. 54, 19-48.

604 Sick, K. 1965*b*. Haemoglobin polymorphism of cod in North Sea and North Atlantic Ocean.
605 Hereditas-Genetisk A. 54, 49-69.

606 Skjæraasen, J.E., Nilsen, T., Meager, J.J., Herbert, N.A., Moberg, O., Tronci, V., Johansen, T.,
607 Salvanes, A.G.V. 2008. Hypoxic avoidance behaviour in cod (*Gadus morhua* L.): The effect of
608 temperature and haemoglobin genotype. J. Exp. Mar. Biol. Ecol. 358, 70-77.

609 Stearns, S.C. 1992. The Evolution of Life Histories. Oxford University Press. 248 p.

610 Swain, D.P., Kramer, D.L. 1995. Annual variation in temperature selection by Atlantic cod
611 *Gadus morhua* in the southern Gulf of St. Lawrence, Canada, and its relation to population size.
612 Mar. Ecol-Prog. Ser. 116, 11-23.

613 Tao, W.J., Boulding, E.G. 2003. Associations between single nucleotide polymorphisms in
614 candidate genes and growth rate in Arctic charr (*Salvelinus alpinus* L.). Heredity 91 (1), 60-69.

615 Val, A.L. 2000. Organic phosphates in the red blood cells of fish. Comp. Biochem. Physiol. A
616 125 (4), 417-35.

617 Verde, C., Balestrieri, M., Pascale, D.M., Pagnozzi, D., Lecointre, G., Prisco, G. 2006. The
618 oxygen transport system in three species of the boreal fish family Gadidae. J. Biol. Chem. 28
619 (31), 22073-84.

620 Weber, R.E., Jensen, F.B. 1988. Functional adaptations in hemoglobins from ectothermic
621 animals. Annu. Rev. Physiol. 50, 161-79.

622 Wennevik, V., Jørstad, K.E., Dahle, G., Fevolden, S-E. 2008. Mixed stock analysis and the
623 power of different classes of molecular markers in discriminating coastal and oceanic Atlantic

624 cod (*Gadus morhua* L.) on the Lofoten spawning grounds, Northern Norway. Hydrobiologia
625 606, 7-25.

626 Wilkins, N.P. 1967. Polymorphism of whole blood proteins in the cod (*Gadus morhua* L.). Cons.
627 Perm. Int. Explor. Mer. 31 (1), 77-88.

628 Wilkins, N.P. 1969. Haemoglobin polymorphism in cod, whiting and Pollack in Scottish waters.
629 Cons. Perm. Int. Explor. Mer. 161, 60-63.

630

631 **Figure captions**

632 Fig. 1 Monthly *HbI*$^{1}$ allele frequencies along the Norwegian coast. The *HbI*$^{1}$ frequencies are
633 based on reported cod blood sampling from a wide range of authors (see Supplementary Material
634 for a complete table with *HbI*$^{1}$ frequencies).

635 Fig. 2 Monthly *HbI*$^{1}$ allele frequencies in the North Sea. The *HbI*$^{1}$ frequencies are based on
636 reports by a wide range of authors, while the annual average bottom temperatures are adopted
637 from other sources as well (see Supplementary Material for a complete table with *HbI*$^{1}$
638 frequencies and the matching bottom temperatures).

639 Fig. 3 *HbI*$^{1}$ frequency as a function of bottom temperature. The *HbI*$^{1}$ frequencies are based on
640 reports by a wide range of authors, while the annual average bottom temperatures are adopted
641 from other sources as well (see Supplementary Material for a complete table with *HbI*$^{1}$
642 frequencies and the matching bottom temperatures).

643 Fig. 4 *HbI*$^{1}$ frequencies of cod in the North Atlantic. *HbI*$^{1}$ frequencies are shown with different-
644 sized circles and bottom temperatures are shown as squares with a colour gradient. Both
645 variables are based on the values shown in table 1. For simplicity, some of the values have been
646 aggregated according to area.

## Tables

Table 1 *HbI*[1] frequencies for different geographical areas

Tabulated *HbI*[1] values are averages for a given period and area. The data are aggregated in the most suitable way to reduce the number of samples, thus the exact same values may not be found in the original source. The latitude and longitude correspond to the approximate midpoint of each area. Bottom temperatures are averages for the period 1960-2010 taken from ICES.

| Year | Month | HbI[1] freq. | Latitude | Longitude | Bottom temperature | Source |
|---|---|---|---|---|---|---|
| North America | | | | | | |
| 1967-81 | - | 0.05 | 54 | -57 | 0 | Jamieson and Birley 1989 |
| 1967-81 | - | 0.06 | 49 | -56 | 0 | Jamieson and Birley 1989 |
| 1962 | 6 | 0.04 | 47 | -52 | 0 | Sick 1965*b* |
| 1967-81 | - | 0.1 | 47 | -60 | 1.75 | Jamieson and Birley 1989 |
| 1967-81 | - | 0.07 | 42 | -68 | 8 | Jamieson and Birley 1989 |
| 1963 | 1 | 0.07 | 39 | -75 | 8 | Sick 1965*b* |
| 1962 | 3 | 0.08 | 41 | -70 | 8 | Sick 1965*b* |
| Greenland | | | | | | |
| 1969-74 | 3-7 | 0.09 | 74 | -26 | 2 | Jamieson and Birley 1989 |
| 1961/63 | 9 | 0.01 | 66.5 | -50.5 | 2 | Sick 1965*b* |
| Barents Sea | | | | | | |
| 1993 | 1 | 0.14 | 68 | 41 | 4 | Fyhn *et al.* 1994 |
| 1993 | 1 | 0.08 | 70 | 40 | 4 | Jørstad and Nævdal 1993 |
| 1964-67 | 1/11 | 0.11 | 72 | 26.5 | 4 | Møller 1968 |

| | | | | | | |
|---|---|---|---|---|---|---|
| White Sea | | | | | | |
| 1974-80 | 6-8 | 0.16 | 65 | 35.5 | 2 | Karpov *et al.* 1984 |
| 1974-80 | 9-11 | 0.16 | 65 | 35.5 | 2 | Karpov *et al.* 1984 |
| Baltic Sea | | | | | | |
| 1962 | 5 | 0.04 | 63 | 18 | 3 | Sick 1965*a* |
| 1962 | 10 | 0.01 | 63 | 18 | 3 | Sick 1965*a* |
| 1962 | 5 | 0.01 | 60 | 19 | 4 | Sick 1965*a* |
| 1999 | 4 | 0.01 | 54.5 | 18.5 | 5 | Pörtner *et al.* 2001 |
| 1999 | 4 | 0.1 | 54 | 14.5 | 5 | Pörtner *et al.* 2001 |
| 1999 | 4 | 0.61 | 54 | 10.5 | 5 | Pörtner *et al.* 2001 |
| 1968 | 4/7/8 | 0.27 | 54.5 | 14.5 | 5 | Jamieson and Otterlind 1971 |
| 1968 | 8 | 0.15 | 57 | 18.5 | 5 | Jamieson and Otterlind 1971 |
| 1968 | 12 | 0.43 | 55 | 14.5 | 5 | Jamieson and Otterlind 1971 |
| 1961-62 | 2-8 | 0.21 | 54.5 | 14 | 5 | Sick 1965*a* |
| 1968 | 7-8 | 0.34 | 54.5 | 13.5 | 5 | Jamieson and Birley 1989 |
| 1962 | 3 | 0.03 | 55 | 19 | 5 | Sick 1965*a* |
| 1961 | 11 | 0.04 | 56 | 18.5 | 5 | Sick 1965*a* |
| 1961 | 10 | 0.03 | 59 | 21 | 5 | Sick 1965*a* |
| 1968 | 10-12 | 0.51 | 57 | 18.5 | 5 | Jamieson and Otterlind |
| 1961-63 | 10-11 | 0.3 | 54.5 | 14 | 5 | Sick 1965*a* |
| 1968 | 9-12 | 0.44 | 54.5 | 13.5 | 5 | Jamieson and Birley 1989 |
| Iceland | | | | | | |
| 1968 | 12 | 0.4 | 66.5 | -17 | 5.8 | Jamieson and Jonsson 1971 |
| 1969-74 | 2-12 | 0.19 | 66.5 | -22.5 | 5.8 | Jamieson and Birley 1989 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 1969-74 | 2-12 | 0.11 | 66 | -23 | 5.8 | Jamieson and Birley 1989 |
| 1969-74 | 2-12 | 0.32 | 66.5 | -20 | 5.8 | Jamieson and Birley 1989 |
| 1969-74 | 2-12 | 0.3 | 66.5 | -15.5 | 5.8 | Jamieson and Birley 1989 |
| 1969-74 | 2-12 | 0.61 | 63.5 | -20 | 5.8 | Jamieson and Birley 1989 |
| 1969-74 | 2-12 | 0.09 | 63 | -22 | 5.8 | Jamieson and Birley 1989 |
| 1969-74 | 2-12 | 0.1 | 64 | -13 | 5.8 | Jamieson and Birley 1989 |
| 1968-69 | 3/4/6 | 0.16 | 64.5 | -21.5 | 5.8 | Jamieson and Jonsson 1971 |
| Danish waters | | | | | | |
| 1961 | 8 | 0.63 | 57 | 11 | 7 | Sick 1965*a* |
| 1968 | 12 | 0.62 | 55 | 12 | 7 | Jamieson and Otterlind 1971 |
| 1961 | 2/4/8 | 0.61 | 55.5 | 11 | 7 | Sick 1965*a* |
| 1993 | 6 | 0.59 | 58 | 8 | 7 | Fyhn *et al.* 1994 |
| 1961 | 6-8 | 0.66 | 57 | 9 | 7 | Sick 1965*a* |
| 1961-62 | 3/8 | 0.62 | 54.5 | 11 | 7 | Sick 1965*a* |
| 1961 | 5 | 0.62 | 55.5 | 12 | 7 | Jamieson and Birley 1989 |
| 1961 | 11 | 0.58 | 55.5 | 11 | 7 | Sick 1965*a* |
| 1961 | 11 | 0.62 | 55 | 12 | 7 | Sick 1965*a* |
| 1994 | 10 | 0.59 | 55 | 10 | 7 | Husebø *et al.* 2004 |
| 1994 | 10 | 0.67 | 55 | 12 | 7 | Husebø *et al.* 2004 |
| Norwegian coast | | | | | | |
| 1985-90 | - | 0.25 | 68.5 | 17.5 | 7.13 | Dahle and Jørstad 1993 |
| 2002 | 4 | 0.36 | 68 | 16 | 7.13 | Dahle and Jørstad 1993 |
| 1992 | 4 | 0.22 | 62 | 5 | 7.13 | Fyhn *et al.* 1994 |
| 1994 | 4 | 0.39 | 63 | 5.5 | 7.13 | Husebø *et al.* 2004 |
| 1965-67 | 2-4 | 0.17 | 67.5 | 14.5 | 7.13 | Møller 1968 |

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|---|---|---|---|---|---|---|
| 1962 | 2-3 | 0.52 | 59 | 5 | 7.13 | Frydenberg *et al.* 1965 |
| 1961-62 | 2-3 | 0.63 | 58 | 7 | 7.13 | Frydenberg *et al.* 1965 |
| 1962 | 2-3 | 0.12 | 67.5 | 13.5 | 7.13 | Frydenberg *et al.* 1965 |
| 1962 | 3 | 0.49 | 63.5 | 11.3 | 7.13 | Frydenberg *et al.* 1965 |
| 1963 | 3 | 0.1 | 70.5 | 31.5 | 7.13 | Frydenberg *et al.* 1965 |
| 1994 | 3 | 0.22 | 68.5 | 16.5 | 7.13 | Husebø *et al.* 2004 |
| 1965-66 | 3 | 0.14 | 67 | 10.5 | 7.13 | Møller 1968 |
| 1965-66 | 3 | 0.23 | 69.5 | 17.5 | 7.13 | Møller 1968 |
| 1965-66 | 3 | 0.24 | 70.5 | 24.5 | 7.13 | Møller 1968 |
| 1987-90 | 3-4 | 0.21 | 67.5 | 14 | 7.13 | Dahle and Jørstad 1993 |
| 2002 | 3-4 | 0.23 | 71 | 27 | 7.13 | Dahle and Jørstad 1993 |
| 1992 | 3-4 | 0.12 | 67 | 12 | 7.13 | Fyhn *et al.* 1994 |
| 1994 | 3-4 | 0.6 | 60 | 4 | 7.13 | Husebø *et al.* 2004 |
| 1978/84 | 3-4 | 0.47 | 63.5 | 11 | 7.13 | Mork and Sundnes 1985 |
| 1966-67 | 3-4 | 0.27 | 62.5 | 6 | 7.13 | Møller |
| 1995-97 | 3-4 | 0.23 | 67.5 | 13.5 | 7.13 | Nordeide and Båmstedt 1998 |
| 2002-03 | 3-4 | 0.2 | 68 | 13.5 | 7.13 | Wennevik *et al.* 2008 |
| 1992-93 | 4-5 | 0.57 | 60 | 6 | 7.13 | Fyhn *et al.* 1994 |
| 1962 | 1 | 0.5 | 62 | 5 | 7.13 | Frydenberg *et al.* 1965 |
| 1962 | 1 | 0.64 | 58.5 | 9 | 7.13 | Frydenberg *et al.* 1965 |
| 2002 | 3-5 | 0.63 | 60 | 4 | 7.13 | Dahle and Jørstad 1993 |
| 1994 | 5 | 0.6 | 58.5 | 9 | 7.13 | Husebø *et al.* 2004 |
| 1989-91 | 5-8 | 0.61 | 59 | 9.5 | 7.13 | Gjøsæter *et al.* 1992 |
| 1977-83 | 10 | 0.5 | 63.5 | 11 | 7.13 | Mork and Sundnes 1985 |
| 1962 | 9 | 0.42 | 65 | 11 | 7.13 | Frydenberg *et al.* 1965 |
| 1961-62 | 9 | 0.44 | 62.5 | 7 | 7.13 | Frydenberg *et al.* 1965 |
| 1994 | 9 | 0.58 | 58.5 | 9 | 7.13 | Husebø *et al.* 2004 |
| 1986-90 | 9-1 | 0.6 | 59 | 9.5 | 7.13 | Gjøsæter *et al.* 1992 |
| 2001 | 9-11 | 0.43 | 67 | 13 | 7.13 | Dahle *et al.* 2006 |
| Faeroe Waters | 4 | 0.07 | 61.5 | -6 | 7.4 | Sick 1965*b* |

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|---|---|---|---|---|---|---|
| 1961/63 | | | | | | |
| 1992 | 1 | 0.08 | 62 | -7 | 7.4 | Fyhn *et al.* 1965 |
| 1961-62 | 7-8 | 0.02 | 65.5 | -21.5 | 7.4 | Sick 1965*b* |
| 1971/1976 | 3-6 | 0.06 | 62 | -7 | 7.4 | Jamieson and Birley 1989 |
| 1971/1976 | 3-6 | 0.19 | 60.5 | -9 | 7.4 | Jamieson and Birley 1989 |
| 1961-62 | 5/8 | 0.02 | 64 | -20 | 7.4 | Sick 1965*b* |
| 1970/1976 | - | 0.6 | 0.35 | -6 | 9.57 | Jamieson and Birley 1989 |
| 1971-72/74/77 | 3 | 0.57 | 53.5 | -5 | 10.56 | Jamieson and Birley 1989 |
| 1966 | | | | | | |
| North Sea | | | | | | |
| | 4/8 | 0.58 | 60 | -1 | 7.62 | Wilkins 1969 |
| North Sea | | | | | | |
| | | | | | | Jamieson and Thompson |
| 1970 | 1 | 0.44 | 58 | 3 | 7.62 | 1972 |
| 1967-81 | 7 | 0.58 | 59.5 | 1.5 | 7.62 | Jamieson and Birley 1989 |
| | | | | | | Jamieson and Thompson |
| 1970 | 6 | 0.63 | 58 | -5 | 7.62 | 1972 |
| 1965 | 4 | 0.55 | 57 | -2 | 8.56 | Wilkins 1967 |
| | | | | | | Jamieson and Thompson |
| 1969 | 12 | 0.68 | 54 | 2 | 8.56 | 1972 |
| 1999 | 2 | 0.66 | 55.5 | 6.5 | 8.56 | Pörtner |
| 1966 | 2 | 0.54 | 57 | -2 | 8.56 | Wilkins 1967 |
| 1966-67 | 2/4/6 | 0.6 | 56 | -4.5 | 8.56 | Wilkins 1969 |
| 1967-81 | 2-3 | 0.67 | 55 | 2 | 8.56 | Jamieson and Birley 1989 |
| 1961-63 | 2-9 | 0.62 | 55.5 | 6.5 | 8.56 | Sick 1965*b* |
| 1967-81 | 1/11 | 0.67 | 59.5 | 1.5 | 8.56 | Jamieson and Birley 1989 |
| 1962 | 7 | 0.65 | 57 | 2 | 8.56 | Sick 1965*b* |
| 1966 | 9 | 0.6 | 57 | -2 | 8.56 | Wilkins 1967 |
| 1969-70 | 12-1 | 0.53 | 52 | -2.5 | 11.62 | Jamieson and Thompson |

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|---|---|---|---|---|---|---|
| | | | | | | 1972 |
| | | | | | | Jamieson and Thompson |
| 1968/70 | 2 | 0.66 | 53 | 3 | 11.62 | 1972 |
| 1967-81 | 2-4 | 0.63 | 52 | 2.5 | 11.62 | Jamieson and Birley 1989 |
| 1963 | 1 | 0.72 | 52 | 3 | 11.62 | Sick 1965*b* |
| 1967-81 | 10-12 | 0.6 | 52 | 2.5 | 11.62 | Jamieson and Birley 1989 |
| 1968 | 12 | 0.73 | 50.5 | -2.5 | 12.23 | Jamieson and Thompson |
| 1966 | 2 | 0.5 | 50 | -4 | 12.23 | Wilkins 1969 |
| 1971 | 3 | 0.6 | 50.5 | 0 | 12.23 | Jamieson and Birley 1989 |

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655 Table 2 Overview of experimental and field studies on cod *HbI*

| Study | Focus | Stage | Area | N | Results | Authors' Comments | Reference |
|---|---|---|---|---|---|---|---|
| Lab | $O_2$-affinity 0-20˚C *In-vitro* | Adults | White Sea | - | *HbI-2/2* have higher $O_2$-affinity <15˚C and *HbI-1/1* >15˚C | | Karpov and Novikov (1981) |
| Field | Maturation | Adults | Trondheims fjord | 276 | *HbI-2/2* males mature earlier<br>*HbI-2/2* females have more developed gonads early in the season | Results are likely secondary effects of growth | Mork *et al.* (1983) |
| Field | Haemat-ocrit | Juv., adults | Trondheims fjord | 149 | *HbI-2/2* males have higher haematocrit<br>*HbI-2/2* juvenile males are larger | Large seasonal temperature fluctuations may influence the result | Mork and Sundnes (1984) |

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| Field | Growth<br>Maturation | Adults | Trondheims fjord and Olso fjord | - | *HbI-2/2* are larger than *HbI-1/1*<br>(males: *HbI-2/2* > 1/2 > 1/1, females: HbI-1/2 > 2/2 > 1/1)<br>Male *HbI-2/2* mature earlier | Size-selective fishing will tend to level out potential genotypic differences | Mork *et al.* (1984*b*) |
| Field | Growth<br>Genotypic frequency | Im-mature | Trondheims fjord | 119 | *HbI-2/2* are largest, *HbI-1/1* smallest<br>Year-class variation | *HbI* unreliable as a population marker | Mork *et al.* (1984*b*) |
| Lab | Growth<br>6, 10, 14˚C | Larvae | Farmed (origin: Bergen) | 100 per exp. | *HbI-2/2* have highest growth rate at all temperatures<br>*HbI-1/1* have lowest | Perhaps due to differences in metabolic capacity | Nævdal *et al.* (1992) |
| Field | Growth | Juv., adults | Skagerrak | 12-1300 | No differences | The benefit of being of a particular genotype may be temperature dependent | Gjøsæter *et al.* (1992) |
| Field | Growth | Juv.<br>4-24 months | Tromsø/ Bergen and Barents Sea | Ca. 2500 | Only significant differences (*HbI-2/2* larger) in 1 out of 10 samples (consisting of 281 fish) | | Jørstad and Nævdal (1994) |
| Lab | Growth<br>12, 14, 16, 18, 20$^0$C | Juv. | Bergen | 50 per exp. | A non-significant tendency for higher growth in *HbI-2/2* at 12$^0$C | Perhaps significant differences if | Glover *et al.* (1997) |

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| | 3 diff. light regimes | | | | and for *HbI-1/1* at higher temperatures | the $O_2$ sat. level was less favourable | |
| Lab | $O_2$-affinity 10, 15 and 20˚C *In-vitro* | Adults | Bergen | 60-70 per exp. | *HbI-2/2* have higher $O_2$-affinity at 10-15˚C and *HbI-1/1* at 20˚C | Perhaps due to differences in metabolic capacity | Brix *et al.* (1998) |
| Lab | $O_2$-affinity 5, 10, 16˚C *In-vitro* Hypoxia tolerance Haematocrit | Adults | Øresund, Denmark and Lowestoft, Norway (Haemato-crit) | 40-70 | *HbI-2/2* have higher $O_2$-affinity at 4˚C (acclimation) and at 4 and 16˚C (acute exposure) *HbI-2/2* have lower $S_{crit}$ at 4˚C and *HbI-1/1* at 16˚C *HbI-2/2* have higher haematocrit at 5 and 10˚C | *HbI-2/2* has superior oxygen transport at low temperatures which supports the observed increase in $HbI^2$ with latitude | McFarland (1998) |
| Lab | Competition Feeding behaviour | 1-group | Farmed 6-7˚C (origin: W. Norway) | 29 | *HbI-2/2* have highest capture success and take prey first | When fish have to compete for food *HbI-2/2* have an advantage | Salvanes and Hart (2000) |
| Field/ Lab | Growth (field) $O_2$-affinity (lab) 4, 8˚C *In-vitro* | Adults | North Sea and Baltic Sea | 803: field 30: lab | *HbI-2/2* are largest in the Baltic Sea and smallest in the North Sea *HbI-2/2* had higher $O_2$-affinity at 4˚C | Genetic differences in temperature specific growth rate cannot be overcome by acclimation | Pörtner *et al.* (2001) |
| Lab | Growth | 1- | Farmed | 44 | No differences | Temperature | Colosimo |

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| | $O_2$-affinity 4, 12˚C *In-vitro* | group | (origin: W. Norway and Barents Sea) | and 16 ($O_2$) | Highest $O_2$-affinity at 12˚C irrespective of genotype | drives the aggregation of physiologically similar individuals | *et al.* (2003) |
| Lab | $T_{pref}$ | Juv. | Øresund | 16 | *HbI-2/2*: 8.2±1.5˚C (norm/hypoxia) *HbI-1/1*: 15.4±1.1°C (normoxia) and 9.8±1.8˚C (hypoxia) | A similar decrease in $T_{pref}$ for *HbI-2/2* may occur at $O_2$ levels < 33% | Petersen and Steffensen (2003) |
| Lab | $O_2$-affinity 4, 12˚C *In-vitro* | 1-group | Farmed (origin: W. Norway) | 126 | *HbI-2/2* have higher $O_2$-affinity at 4˚C No differences at 12˚C | Acclimation changes the relative amount of the different Hb components | Brix *et al.* (2004) |
| Lab | Growth | Juv. | Farmed 9-11˚C (origin: W. Norway and Barents Sea) | Ca. 6100 | No differences | | Gjerde *et al.* (2004) |
| Lab | Growth 7, 10, 13, 16˚C and T-steps (16, 13, 10˚C) | Juv. | Farmed 10˚C (origin: Western Norway) | 220 | *HbI-2/2* have higher growth rate at 13-16˚C *HbI-1/1* at 7˚C No differences at 12˚C (most pronounced under T-step exp.) | *HbI-1/1* have lowest $T_{opt}$ (12.5 ˚C) and HbI-1/2 highest (14.5˚C) Perhaps due to differences in metabolic | Imsland *et al.* (2004) |

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|---|---|---|---|---|---|---|---|
| | | | | | | capacity | |
| Lab | Growth (4-16˚C) | Juv. | Farmed + wild (origin: W. Norway and Barents Sea) | 570 | No differences | Not able to distinguish between differences related to family group and genotype | Jørstad *et al.* (2006) |
| Lab | Growth 6, 14˚C Energy retention and feed conversion efficiency | Juv. | Farmed 8˚C (origin: W. Norway) | 306 | No differences in growth *HbI-2/2* have a tendency for highest energy retention and feed conversion efficiency | $T_{pref}$ is not necessarily a good indicator for $T_{opt}$ | Jordan *et al.* (2006) |
| Lab | Hypoxia avoidance behaviour 5, 15˚C | Juv. | Farmed 8-10˚C (origin: Bergen) | 28 | No differences in avoidance response or ventilation rates *HbI-1/1* swam faster than *HbI-2/2* at 15˚C | Overall reduced swimming speed and avoidance of most hypoxic habitat | Skjæraasen *et al.* (2008) |
| Lab | Temp. and hypoxia avoidance | Juv. | Farmed 10˚C (origin: Nova Scotia) | 100-400 | No significant differences but *HbI-1/1* had lower $S_{crit}$ indicating that this genotype is slightly more tolerant to hypoxia *HbI-1/1* grew faster in the first 9 months and had higher mortalities | Differences in growth is likely not caused by reduced SMR or higher metabolic capacity | Gamperl *et al.* (2009) |

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|---|---|---|---|---|---|---|---|
| Lab | Hypoxia/stress response 5, 10 and 15˚C | Juv. | Øresund | 50-60 | *HbI-1/1* have higher plasma cortisol and lactate levels indicative of an increased level of stress | *HbI-2/2* are perhaps more tolerant to hypoxia | Methling *et al*. (2010) |
| Lab | $T_{pref}$ | Juv. | Øresund | 24 | *HbI-2/2*: 8.9±0.2°C<br>*HbI-1/1*: 11.0±0.6°C<br>*HbI-1/2* have a wider range of $T_{pref}$ | *HbI-1/2* are likely more flexible | Behrens *et al.* (2012) |

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## Figures

Fig. 1 Monthly *HbI*$^{1}$ allele frequencies along the Norwegian coast

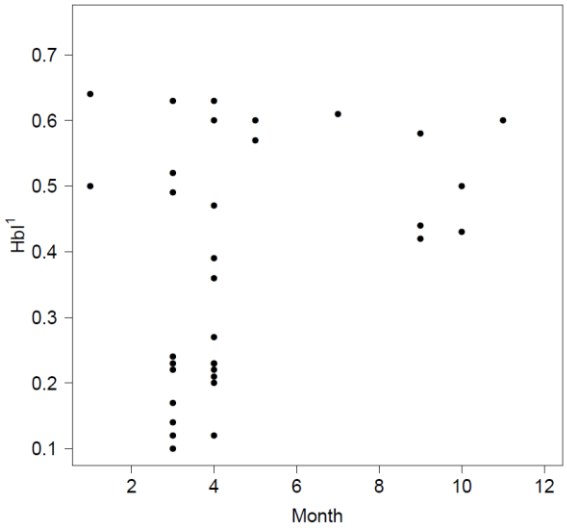
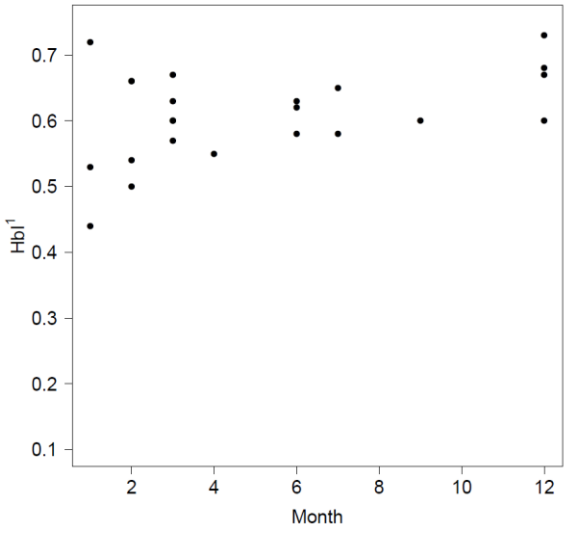
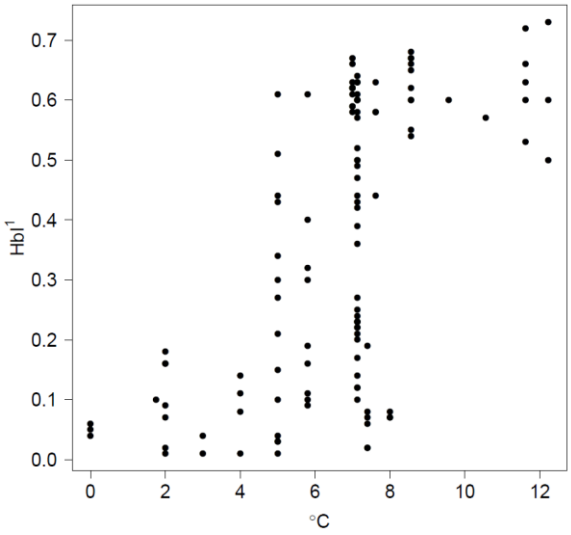



Fig. 2 Monthly *HbI*$^{1}$ allele frequencies in the North Sea



664 Fig. 3 *HbI*[1] frequency as a function of bottom temperature

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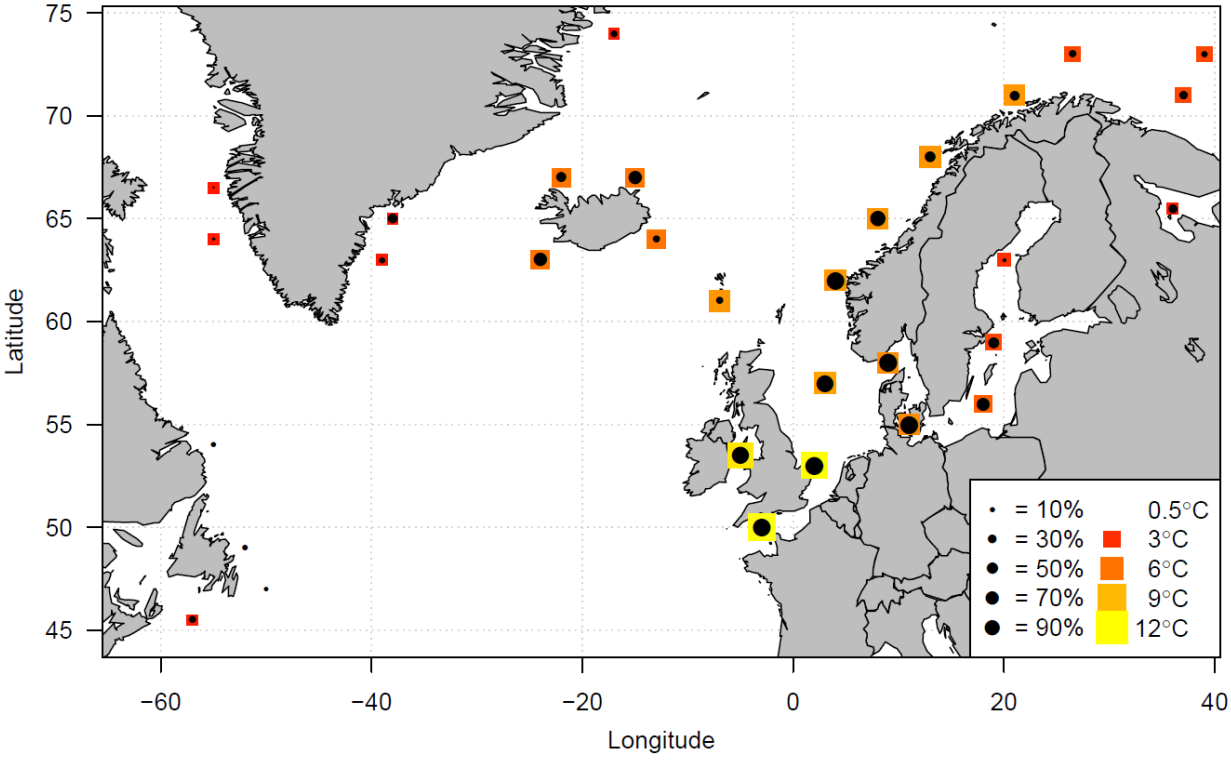
666 Fig. 4 *HbI*[1] frequencies of cod in the North Atlantic

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